

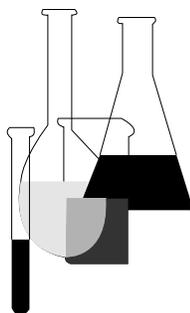


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# Ecological Effects Test Guidelines

## OPPTS 850.4250

### Vegetative Vigor, Tier II



**“Public Draft”**

## INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

**Public Draft Access Information:** This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher ([gopher.epa.gov](http://gopher.epa.gov)) under the heading “Environmental Test Methods and Guidelines” or in paper by contacting the OPP Public Docket at (703) 305-5805 or by e-mail: [guidelines@epamail.epa.gov](mailto:guidelines@epamail.epa.gov).

**To Submit Comments:** Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: [guidelines@epamail.epa.gov](mailto:guidelines@epamail.epa.gov).

**Final Guideline Release:** This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: [fedbbs.access.gpo.gov](http://fedbbs.access.gpo.gov) (IP 162.140.64.19), or call 202-512-0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher ([gopher.epa.gov](http://gopher.epa.gov)) under the heading “Environmental Test Methods and Guidelines.”

**OPPTS 850.4250 Vegetative vigor, tier II.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 797.2750 Seed Germination/Root Elongation Toxicity Test and OPP guideline 123–1 Seed Germination/Seedling Emergence and Vegetative Vigor (Tier II) (Pesticide Assessment Guidelines, Subdivision J—Hazard Evaluation; Nontarget Plants), EPA report 540/09–82–020, 1982.

(b) **Tier II—vegetative vigor dose response testing.** This guideline should be used in conjunction with OPPTS guideline 850.4000, Background—Nontarget Plant Testing, which provides general information and overall guidance for the nontarget plants test guidelines.

(1) **Objective.** (i) This guideline should be used in conjunction with OPPTS guideline 850.4000, Background—Nontarget plant testing, which provides general information and overall guidance for the Group D guidelines of the 850 series. The terrestrial nontarget plant phytotoxicity tests are laboratory tests that evaluate the acute toxicity of pesticides to 10 crop plants. These studies evaluate the effects of multiple dosage levels on plant growth, using less than the maximum label rate with dosages in a geometric progression of no more than twofold, and with subtoxic (<EC50) and nontoxic (no observable effect level, NOEL) concentrations. The typical end-use product is used, and results are reported as EC25, EC50 and NOEC (or EC05) values in pounds of active ingredient (AI) per acre. Parameters measured include plant height, plant dry weight, root length and root dry weight (if a root active compound), and observed phytotoxicity. The results are used to establish acute toxicity levels, compare with measured or estimated environmental concentrations, and to indicate if further testing at a higher tier is necessary.

(ii) Nontarget plant phytotoxicity data are used to assess the potential hazard of chemicals to nontarget plants—terrestrial, semiaquatic, or aquatic. Nontarget plants include plants outside the area of intended application (which would include food and cover vegetation for animals, food, fiber, fuel, and ornamental plants for man, and endangered and threatened plants). Phytotoxicity data are occasionally requested in order to assess the potential hazard of certain pesticides to plants within the treatment area (target area testing). Terrestrial plants serve as food and shelter for fish and wildlife, help control erosion, and serve as air pollution filters. Shoreline vegetation buffers reduce siltation and provide an environment in which aquatic invertebrates can contribute to the food supply of fish, reptiles, and amphibians. Vegetation adjacent to streams serves to regulate water temperature which in turn contributes to improved aquatic life. De-

caying vegetation provides nutrients essential for the aquatic food chain. Hedgerows, woodlots, and other similar nontarget areas provide food and cover to mammalian and avian species.

(iii) Nontarget plant phytotoxicity data are routinely used to conduct ecological risk assessments for the registration and reregistration of pesticides. These data are also useful in our assessment of potential hazards to endangered/threatened plant species listed by the Department of Interior, Fish and Wildlife service.

(iv) Tier II tests are initiated following a determination that a greater than 25 percent adverse effect occurred in the Tier I vegetative vigor study for one or more plant species. If less than a 25 percent detrimental effect or response to all tested species is noted in the vegetative vigor study, no higher tier testing is ordinarily required. Under FIFRA, if the pesticide is a known phytotoxicant, terrestrial plant testing begins with Tier II. Unique Agency concerns arising from Special Review issues, pesticide contamination cases, 6(a)(2) incidents (adverse effects reporting), published literature, or other public sources may result in requests for additional tests.

(c) **Test methods**—(1) **Test facility/location/test conditions.** Dose response vegetative vigor tests can be conducted in the greenhouse or in small field plots. Report soil type and texture, soil  $K_d$  values, soil  $K_{oc}$  values, and soil pH. Environmental conditions during the test should be recorded daily—light intensity, air temperature, humidity, photoperiods, thermoperiods, watering schedules and methods (rainfall if field test), and pest conditions are to be recorded.

(2) **Test substance.** Refer to 40 CFR part 160 for test substance requirements. Use of TEP instead of TGAI is preferred for all nontarget plant phytotoxicity tests, using the TEP with the highest percent active ingredient and/or the one most commonly used.

(3) **Controls/solvents/additives and other pesticide treatments.** Pesticide treatments other than the test pesticide should be avoided. Mechanical, cultural, and biological pest control methods are suggested. If solvents or other pesticides are used in the test, the registrant must show that the solvent/pesticide is not toxic to the test species and that no synergistic or antagonistic interactions with the test pesticide exists (additional test data). To demonstrate solvent or adjuvant activity, a separate set of control plants (set aside for this purpose at the beginning of the experiment) can be treated with the solvent/pesticide using the highest dosage. A negative control is still required. If the solvent/pesticide controls and the negative control are contaminated with the test chemical, the study should most likely be repeated. If the solvent control is contaminated with the test chemical and the negative control is not (and visa versa), the study may not be invalid if zero percent toxicity occurred in the negative control

and at the lowest dose tested. Within a given study, all test organisms including the controls should be from the same source. To prevent bias, a system of random assignment of the test plants to test and control groups is required. If adjuvants are recommended on a TEP label, a representative adjuvant within each class (anionic, ionic, nonionic, etc.) must be used in the test.

(4) **Equipment.** (i) All equipment used in conducting the test, including equipment used to prepare and administer the test substance, and equipment to maintain and record environmental conditions, should be of such design and capacity that tests involving this equipment can be conducted in a reliable and scientific manner. Equipment should be inspected, cleaned, and maintained regularly, and be properly calibrated. Acceptable types of sprays and foliar spray methods are found in paragraph (f)(5) of this guideline. Computerized belt sprayers are also acceptable.

(ii) The application equipment used in testing products in small field plot studies should be designed to simulate conventional farm equipment using the basic components of commercial application equipment in the design of the small-plot equipment. For example, nozzle types, sizes, and arrangements on small plot sprayers can be identical to those used by growers on commercial ground sprayers. Specific details as to descriptions of equipment design, adjustment, and operation should be provided in test reports.

(5) **Dosages.** (i) At least five dosages should be tested.

(ii) The dosages should include a subtoxic (<EC50) and a nontoxic concentration.

(iii) The dosages should be of geometric progressions of no more than fourfold. A twofold progression is preferred: An example of a twofold series is 0.1, 0.2, 0.4, 0.8, and 1.6 kg/ha. However, threefold and fourfold progressions are acceptable with the proviso that the test be extended for the most sensitive test species if the no-effect level (or EC05) has not been achieved. The test must define the response curve so that accurate EC50, EC25, and NOEC (or EC05) values can be obtained. The lack of a no-effect level is not critical as long as the slope is adequate for calculation of valid EC50, EC25, and EC05 values from the most sensitive endpoints (e.g. dry shoot weight, dry root weight, shoot height). The lowest test level and the NOEC should not be greater than the EC25 value.

(iv) Dosages should be reported in units of active ingredient or acid equivalent as appropriate. Rates may be expressed as units of ingredient per unit of land area to be treated, units of concentration (such as parts per million), units per flow rate, or units of ingredient per unit volume applied to obtain a specified degree of foliage coverage (such as “to run-off”).

(v) If a pesticide (FIFRA) is applied more than once within a year or growing season, each rate and the interval between applications should be indicated. If products are applied in a tank mixture or are applied serially, rates and intervals, as appropriate, should be reported with identification and formulation for each product.

(vi) If conducting tests under TSCA, the test chemical is applied daily with each watering for the duration of the study.

(6) **Plant test species.** (i) At least three replicates, each with 10 plants, should be tested per dose level. Larger populations and more replicates of certain plants with low germination may be needed to increase the statistical significance of the test.

(ii) Healthy plants must be used. Pesticide treated seeds should be avoided. The Agency should be consulted prior to test initiation if seed treatments other than steam, a weak hypochlorite solution (recommended by Environment Canada), captan, or thiram are used. Captan and thiram seed treatments are the only approved pesticide seed treatments (noninteractive with most other pesticides). Steam sterilization of soil and seeds is the recommended procedure for killing pathogens, fungi, and insects on seeds and in soil media. Some methods used to remove seed treatments include a weak methanol solution, detergents, and hypochlorite solution rinsing. When unapproved pesticide seed treatments are used in a study, it is the responsibility of the laboratory conducting the test to show that no synergistic or antagonistic interactions occur between or among the various pesticides in the test.

(iii) Ten plant species must be tested. The following plant species and groups are recommended:

(A) Dicotyledoneae: Six species of at least four families, one species of which is soybean (*Glycine max*) and a second of which is a root crop.

(B) Monocotyledoneae: Four species of at least two families, one species of which is corn (*Zea mays*).

(iv) Of the 10 test plants, 3 must be tested: corn (*Zea mays*), soybean (*Glycine max*), and a root crop such as carrot (*Ducus carotta*), onion (*Allium cepa*), beet (*Beta vulgaris*), or sugarbeet (*Beta vulgaris*). The other seven test species might include: tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativa*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), oat (*Avena sativa*), and perennial ryegrass (*Lolium perenne*). Substitution of other test species (other crops, weeds controlled, native plants, perennials, woody species) is encouraged when species sensitivity to the test compound is known ahead of time. Endangered or threatened species as determined by the Endangered Species Act of 1973 (Public Law 93-205) may not be used without permission from the Fish and Wildlife Service.

(7) **Support media and pesticide dosing method.** (i) Plants may be grown in pots using a sterilized standardized soil that consists of primarily sandy loam, loamy sand, loamy clay, or clay loam soil that contains up to 3 percent organic matter. Glass beads, rockwool, and 100 percent acid washed sand are not recommended.

(ii) Test methods and protocols for hydroponic tests should be submitted to the Agency for review prior to test initiation.

(iii) The pesticide is sprayed onto the plant foliage ensuring even and thorough contact with plant surfaces.

(8) **Test containers.** Test containers should be nonporous to prevent absorption of the test material. Do not use peat or clay. Containers should be thoroughly cleaned prior to use. A dichromate solution should not be used to clean containers.

(9) **Test parameters.** (i) Carbon dioxide level should be maintained at  $350 \pm 50$  ppm.

(ii) Relative humidity should approach  $70 \pm 5$  percent during light periods and 90 percent during dark periods.

(iii) Irradiation measured at 1 m from the source, at  $350 \pm 50$   $\mu\text{E}/\text{m}^2$  sec at 400 to 700 nm.

(iv) Photoperiods of 16 h light and 8 h darkness.

(v) Day/night temperatures at  $25/20 \pm 3$  °C.

(vi) Half strength modified Hoagland nutrient solution may be used as nutrient media.

(10) **Watering methods.** Bottom watering of test containers is preferred, however, top watering under the foliage can also be used to prevent washing pesticide off foliage.

(11) **Plant density.** The number of plants per pot is left to the discretion of the laboratory conducting the test, for example: One or two corn, soybean, tomato, cucumber, or sugarbeet plants in a 6-inch container, three rape, or pea plants in a 6-inch container, and a maximum of six onion, wheat, or other small grains in a 6-inch container. The test conditions should approximate those optimal conditions for the species and varieties tested (cool and warm season plants should be tested separately).

(12) **Test duration/end-points.** (i) The test material is applied 2 to 4 weeks after the plants have emerged from the soil. During the minimum 14-day observation period following chemical application, the plants are observed every 7 days (or more frequently) for visual phytotoxicity, total dead plants, and shoot height. At the end of the study, final shoot heights and phytotoxicity measurements are taken prior to harvesting of the above

and below-ground portions for dry weight measurements (root dry weight and root length measurements are optional depending on the chemicals mode-of-action information, incident reports, literature, etc.). If abnormal symptoms occur, the observations should be continued until the plant dies or fully recovers. The individual test species responses to environmental conditions, the mode-of-action of the pesticide, and the speed of uptake of the pesticide by the test plants are factors that affect study duration. The registrant is expected to extend the duration of the study as dictated by these factors. All dead plants should be measured, weighed, and observed and included in the statistical analysis.

(ii) Observations should include all variations, either inhibitory or stimulatory, between the treated test organisms and the untreated control test organisms. Such variations may be phytotoxic symptoms (chlorosis, necrosis, and wilting), formative (leaf and stem deformation) effects, and/or growth and development rates. Observations should include the stage of development and dates when adverse results occurred and subsided or recovered. Any lack of effects by the pesticide should also be reported.

(iii) Include actual counts, weights, and other measurements for each plant, replicate and variable. Uniform scoring procedures should be used to evaluate the observable toxic responses. Such data should include the actual values used to determine any percentages of effects. Raw data (chromatographs, field reports, and analysis data) may also be included to substantiate the basic data that are required.

(d) **Reporting requirements**—(1) **General.** Refer to 40 CFR 160.185, subpart J, for reporting requirements. Report should include name of laboratory or test location, personnel information, test substance information, test procedures, materials, methods, results, and analysis of data in tabular summary form. Statistical methods must be described. Any available information on mode-of-action (biochemical) and resultant plant effects should be included. Either the metric system or the U.S. Standard Measures (to preclude extensive conversion to the metric system) may be used in test reports. The two systems cannot be mixed (e.g., grams per square feet). The English language must be used in all test reports; English translations must be provided with foreign language reports.

(2) **Test report.** The test report should include the following information:

(i) Severity of phytotoxicity (percent or rating), abnormal changes in growth and development, and/or abnormal changes in plant morphology as compared to the untreated control.

(ii) Tabulation of the results indicating the percentage effect level for each species as compared to untreated control plants for each test parameter (height, weight, etc.).

(iii) Data on plant dry weight, plant shoot height, root dry weight, root length/volume, number of dead plants, height or other growth parameters are recommended test endpoints.

(iv) Report the actual dates of the studies including dates of initiation (planting, transplanting, and cultural practices), applications, observations, and harvest.

(v) Electronic transfer of test data on disc is encouraged to reduce review time.

(2) **Statistics.** When test results such as efficacy, phytotoxicity, or yield indicate adverse effects on crops and other nontarget test organisms, statistical analysis is required to evaluate the responses. The statistical analysis should consist of:

(i) The tabulation of data for the most sensitive endpoint (plant height, plant dry weight, visual phytotoxicity, etc.) for each plant species tested at each treatment level for each test.

(ii) The determination of 25 and 50 percent detrimental effect levels (EC25, EC50) and the 95 percent confidence limits, where possible, for each (Probit Analysis, Bruce/Versteeg).

(iii) The estimated NOEC (or EC05) and LOEC (Williams Test).

(e) **Special test requirements.** In addition to the data required by this guideline, data from other tests may be required by the Agency for making judgments regarding safety to nontarget plants such as additional test species, life-cycle tests, and monitoring studies. Such data will be required where there are special concerns identified in the literature, 6(a)(2) (adverse effects reporting) or incident action, a unique use pattern, or a unique chemical property.

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Boutin, C. et al. Proposed Guideline For Registration Of Chemical Pesticides: Nontarget plant testing and evaluation. Tech. Rpt. Series No. 145, Canadian Wildlife Service, Environment Canada, pp. 1–91 (1993).

(2) Bruce, R.D. and D.J. Versteeg. A Statistical Procedure For Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry* 11:1485–1494 (1992).

(3) Gulley, D.D. et al. Toxstat. Release 3.0. University of Wyoming, Laramie, WY (1989).

(4) EPA. Nontarget Plants: Seed Germination/Seedling Emergence—Tiers I and II. EPA 540/9–86–132 (1986).

(5) EPA. Nontarget Plants: Target Area Testing. EPA 540/9-86-130 (1986).

(6) Stephan, C.E. Methods for calculating an LC50. In F.L. Mayer and J.L. Hamelink, eds., *Aquatic Toxicology and Hazard Evaluation*, STP 634, American Society for Testing and Materials, Philadelphia, PA, pp. 65-84 (1977).

(7) Truelove, B., (ed.) *Research Methods in Weed Science*. Southern Weed Science Society, Auburn Printing Inc., Auburn, AL 36830 (1977).